

Modeling Microbial Consortia as Distributed Metabolic Networks

JOSEPH J. VALLINO

Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Biogeochemistry is the study of how living systems in combination with abiotic reactions process and cycle mass and energy on local, regional, and global scales (Schlesinger, 1997). Understanding how these biogeochemical cycles function and respond to perturbations has become increasingly important, as anthropogenic impacts have significantly altered many of these cycles (Galloway and Cowling, 2002; Houghton et al., 2002). Biogeochemistry is strongly governed by microbial processes, and it appears to closely follow thermodynamic constraints in that electron acceptor (O_2 , NO_3^- , SO_4^{2-} , etc.) utilization closely follows a priori expectations based on energetics (Vallino et al., 1996; Hoehler et al., 1998; Jakobsen and Postma, 1999; Amend and Shock, 2001). Consortia of microorganisms seem to have evolved to exploit chemical potentials wherever they exist in the environment, as manifested by the recent discovery of anaerobic methane oxidation by sulfate (Boetius et al., 2000) or sulfide oxidation by nitrate (Schulz et al., 1999). Three and a half billion years of natural selection have produced living systems capable of degrading most chemical potentials. We may therefore ask: If all ecosystem niche space is filled, is the biogeochemistry we observe in the environment dependent on the organisms that occupy that environment, or is the biogeochemistry determined by fundamental forces, with the evolution of living systems being the implementation of those forces? Recent developments in nonequilibrium thermodynamics (NET) are beginning to support the latter alternative, and advances in

genomics are allowing us to explore microbial consortia in detail. Taking advantage of ideas being suggested by NET, we have developed a modeling framework that views microbial consortia as an inter-species distributed metabolic network. When combined with experimental observations, the model should help us test hypotheses that govern how living systems function.

The main challenge to understanding microbial biogeochemistry is understanding the complex, but mostly cooperative, metabolism that develops among organisms that orchestrate biogeochemical cycles. Consider, for example, the metabolism found in the rumen of ruminant organisms such as cows (Madigan et al., 2000). The ecosystem of the rumen develops dozens of functional groups consisting of hundreds of species that degrade cellulose and starch to many intermediate organic acids and alcohols, as well as to CO_2 , methane, and hydrogen. Many of these organisms cannot survive without the presence of others. For instance, ethanol fermentation to acetate and H_2 is unfavorable due to the accumulation of H_2 , which is also toxic to many organisms. However, if the fermenting organisms are coupled with methanogens (i.e., syntrophy), the overall reactions can proceed, and are very efficient (Jackson and McInerney, 2002). Furthermore, these systems are controllable, hence predictable, as the host organism is able to utilize a food source that it is not capable of digesting without the organisms in its rumen. What governs the development of this biochemistry? Interestingly, no one organism conducts all these biochemical transformations. Instead, the overall metabolism of the system is distributed across hundreds of different microbial species. Yet the system is well coordinated due to multiple levels of organization and multiple levels of proliferation, not by Darwinian selection (Caldwell et al., 1997). Can this enigmatic coordination be explained by nonequilibrium thermodynamics?

E-mail: jvallino@mbl.edu

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Nonequilibrium Thermodynamics

The application of thermodynamics to living systems dates back to Schrödinger (1944) and his examination of the creation of order from disorder. Although at first it appears that living systems violate the second law of thermodynamics in that they synthesize order from disorder, Schrödinger solved this problem by turning to nonequilibrium thermodynamics (NET). In an open system, energy flux from outside the system can reduce the system's internal entropy, which is offset by an equal or greater increase in entropy outside the system. Since Schrödinger, many investigators have extended the application of NET to living systems (Prigogine, 1955; Margalef, 1968; Morowitz, 1968) with many recent advances (Allen, 1985; Johnson, 1988; Schneider, 1988; Wiley, 1988; Choi *et al.*, 1999; Jørgensen *et al.*, 2000; Toussaint and Schneider, 1988). Schneider and Kay (1994) succinctly describe the current restatement of the Second Law:

Thermodynamic systems exhibiting temperature, pressure and chemical equilibrium resist movement away from their equilibrium states. When moved away from a local equilibrium state a system will behave in a way which opposes the applied gradients and moves it back to its local equilibrium attractor. The stronger the applied gradient, the greater the effect of the equilibrium attractor on the system. The more a system is moved from equilibrium, the more sophisticated its mechanisms for resisting being moved from equilibrium. If dynamic and/or kinetic conditions permit, self-organization processes are to be expected. This behaviour is not sensible from a classical second law perspective, but is what is expected given the restated second law. No longer is the emergence of coherent self-organizing structures a surprise, but rather it is an expected response of a system as it attempts to resist and dissipate externally applied gradients which would move the system away from equilibrium.

Dissipative systems need not always operate at an optimum (Ulanowicz, 1997), because perturbations can reduce system organization, which will result in slower gradient degradation. For example, a perturbation of significant magnitude can destabilize the rumen microbial system and kill the host organism. If the feed changes abruptly from cellulose (grasses) to starch (grains), the organization of the microbial system can collapse due to a rapid increase in the bacteria that produce lactic acid, thus causing acidosis. Interestingly, if the feed is gradually changed, destabilization does not occur. Such phenomena are the essence of self-organization, which occurs in many autocatalytic systems (Ulanowicz, 1997).

Simple examples of the restated second law abound. For example, a chamber of gas isolated from any energy gradients soon reaches equilibrium in which the gas molecules are uniformly distributed (*i.e.*, its highest entropy state). If the chamber is placed within a thermal gradient, the molec-

ular gas distribution is no longer uniform, but shows increased order since a higher density of molecules will reside near the cold end of the chamber. Hence, the thermal gradient has lowered the internal entropy of the chamber. Once more, if the thermal gradient is sufficiently strong, a density-driven circulation will develop, further increasing system order, while reducing system entropy. This self-organization can also be seen in hurricanes, which are the organized structures that develop to facilitate degradation of the thermal gradient that has built up between the atmosphere and ocean over the summer. In an analogous manner, living systems are the organized structures that develop to degrade incoming solar radiation and chemical potential. Indeed, by examining emitted thermal radiation, Schneider and Kay (1994) have found that mature forests degrade incoming solar radiation more effectively than early successional forests or arid land.

The observation that ecosystem function may be governed by laws that transcend Darwinian selection is not new. Many theories related to, or derived from, NET have been developed to describe objective functions living systems tend to follow; however, the choice of an appropriate objective function still remains controversial. This is understandable, since current theories of NET apply only to linear approximations in the neighborhood of an equilibrium (Onsager, 1931; Prigogine, 1978). Theories for systems far from equilibrium are still under development. As a result, objective functions that ecosystems might track are numerous, and include optimizations of exergy (Jørgensen, 1994; Nielsen, 1995; Jørgensen *et al.*, 2000) energy (Odum, 1983), ascendancy (Ulanowicz, 1986), power (Odum and Pinkerton, 1955; Odum, 1971), biomass to maintenance (Margalef, 1968), thermodynamic efficiency (Nielsen and Ulanowicz, 2000), or entropy (Prigogine and Nicolis, 1971). As NET theories develop, perhaps many of these observations and theories will be collapsed or falsified.

Our objective is to develop a modeling framework that can be used to test these various objective hypotheses, but we must first have a basis for the model. Johnson (1988) argues that the rotational pattern of the earth converts the rectilinear energy output of the sun into a pulsed energy input to the earth, which induces cyclical energy flows, resonance, and time delays. This allows the energy of the system to be pumped up before it decays back towards ground state. Metabolically, this is what we see. Autotrophs use incoming solar radiation to create chemical potential in the form of redox gradients. Material at equilibrium, H₂O and CO₂, is pumped into a high-energy state by its conversion to O₂ (oxidizing) and glucose (reducing). Heterotrophs return this redox gradient to ground state in a cyclical manner. Real ecosystem biogeochemistry is more complex when we include abiotic reactions, anaerobic environments, alternate electron acceptors and donors, nutrient constraints, and transport limitations. Nevertheless, it is the buildup and

Table 1

Reactions used to model planktonic microbial community.

Reaction	Stoichiometry
C-fixation	$6 \text{ CO}_2 + 24 \text{ H}^+ + 24 \text{ e}^- \rightleftharpoons \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ H}_2\text{O}$
NH_4^+ uptake	$\text{NH}_4^+ + 0.58 \text{ C}_6\text{H}_{12}\text{O}_6 + 0.034 \text{ SO}_4^{2-} + 0.9 \text{ e}^- \rightleftharpoons \text{AA} + 1.9 \text{ H}_2\text{O}$
NO_3^- uptake	$\text{NO}_3^- + 10 \text{ H}^+ + 0.58 \text{ C}_6\text{H}_{12}\text{O}_6 + 0.034 \text{ SO}_4^{2-} + 8.9 \text{ e}^- \rightarrow \text{AA} + 4.9 \text{ H}_2\text{O}$
Nitrification	$\text{NH}_4^+ + 3 \text{ H}_2\text{O} \rightarrow \text{NO}_3^- + 10 \text{ H}^+ + 8 \text{ e}^-$
N_2 fixation	$\frac{1}{2} \text{ N}_2 + 4 \text{ H}^+ + 0.58 \text{ C}_6\text{H}_{12}\text{O}_6 + 0.034 \text{ SO}_4^{2-} + 3.9 \text{ e}^- \rightarrow \text{AA} + 1.9 \text{ H}_2\text{O}$
Photosynthesis	$2 \text{ H}_2\text{O} \rightarrow \text{O}_2 + 4 \text{ H}^+ + 4 \text{ e}^-$
Respiration	$\text{O}_2 + 4 \text{ H}^+ + 4 \text{ e}^- \rightarrow 2 \text{ H}_2\text{O}$
Protein	$\text{AA} + (\text{Protein})_n \rightleftharpoons (\text{Protein})_{n+1} + \text{H}_2\text{O}$
Chlorophyll	$4 \text{ AA} + 6.8 \text{ C}_6\text{H}_{12}\text{O}_6 + \text{Mg}^{2+} + 46.2 \text{ H}^+ + 48.6 \text{ e}^- \rightleftharpoons \text{Chl} + 42.3 \text{ H}_2\text{O} + 0.14 \text{ SO}_4^{2-}$
Lipids	$8 \text{ C}_6\text{H}_{12}\text{O}_6 + \text{PO}_4^{3-} + 92 \text{ H}^+ + 89 \text{ e}^- \rightleftharpoons \text{C}_{48}\text{H}_{96}\text{O}_6\text{P} + 46 \text{ H}_2\text{O}$
Glycogen	$\text{C}_6\text{H}_{12}\text{O}_6 + (\text{C}_6\text{H}_{10}\text{O}_5)_n \rightleftharpoons (\text{C}_6\text{H}_{10}\text{O}_5)_{n+1} + \text{H}_2\text{O}$
DNA/RNA	$4 \text{ AA} + 2 \text{ PO}_4^{3-} \rightleftharpoons \text{DNA} + 0.73 \text{ C}_6\text{H}_{12}\text{O}_6 + 0.14 \text{ SO}_4^{2-} + 1.9 \text{ H}_2\text{O} + 1.9 \text{ H}^+ + 7.5 \text{ e}^-$

Abbreviations: AA, amino acids ($\text{C}_{3.5}\text{H}_{7.1}\text{O}_{1.7}\text{NS}_{0.034}^{0.03+}$); Chl, chlorophyll ($\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$); Protein ($\text{C}_{3.5}\text{H}_{5.1}\text{O}_{0.7}\text{NS}_{0.034}^{0.03+}$); DNA ($\text{C}_{9.6}\text{H}_{14.0}\text{O}_8\text{N}_4\text{P}_2$). Reactions are either reversible (\rightleftharpoons) or irreversible (\rightarrow).

decay of redox potential *via* a distributed metabolism that forms the cornerstone of our approach. We focus on microbial systems because these systems exhibit the greatest degree of metabolic capacity, are responsible for the majority of biogeochemistry on earth, display fast dynamics that allows for practical experiments, and are less susceptible to loss of diversity.

Thermodynamically Constrained Metabolic Biogeochemical Model

A traditional reductionist biogeochemical model would include differential equations for growth of each microbial functional group, equations for Monod-type growth kinetics, and numerous conditional statements to direct the use of

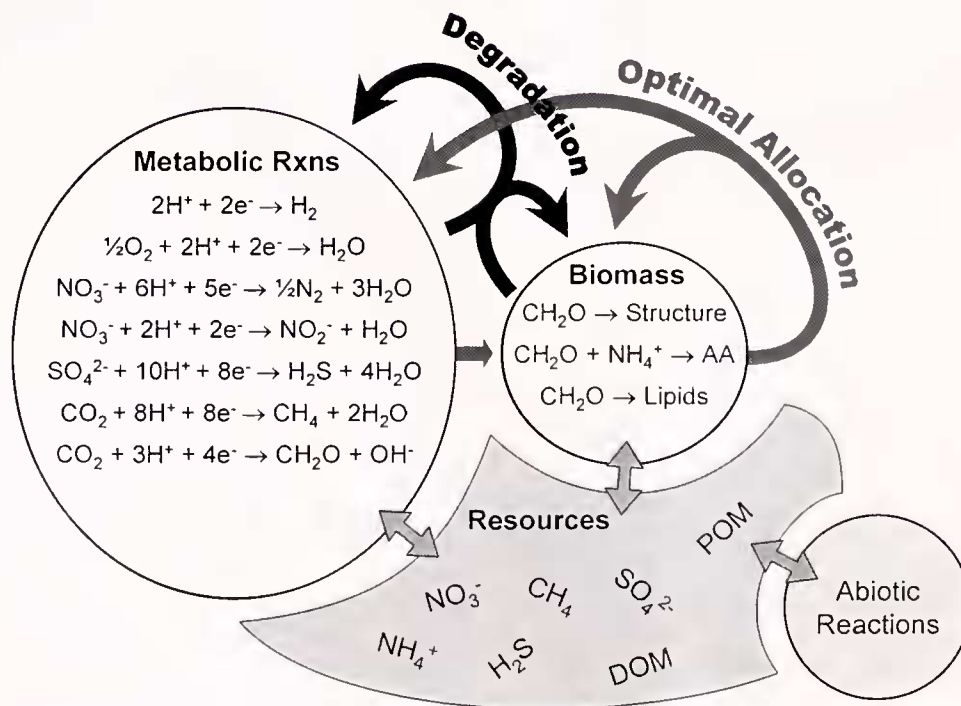


Figure 1. Conceptualization of the metabolic biogeochemistry model. Half reactions lead to production of protein (and other building-block constituents), which is then allocated to reactions governed by an optimization function. Abiotic reactions are incorporated with standard kinetics.

Table 2

Governing equations for the thermodynamically constrained biogeochemistry model

Constraint	Equation
Free energy	$\sum_i r_i \Delta G_i$
Redox	$\sum_i r_i e_i^- = 0$
Biomass composition	$C:N^{\text{Lower}} \leq \frac{\sum R^C(r_i)}{\sum R^N(r_i)} \leq C:N^{\text{Upper}}$
Reaction kinetics	$r_i \leq E_i f_i(S) \quad \forall i$
Enzyme allocation	$\sum_i E_i = E_T$ and $\left. \frac{dE_i}{dt} \right _{\text{Min}} \leq \frac{dE_i}{dt} \leq \left. \frac{dE_i}{dt} \right _{\text{Max}}$
Photosynthesis	$r_p \leq \frac{\text{PAR}(t)}{h} \frac{1}{\Delta G_{tr} [\text{Chl}] + k_{sat}}$
LP Problem	Maximize $\sum_{i,j} R^C(r_i, E_j) + R^N(r_i, E_j)$ r_i, E_j

Definitions: r_i are reaction rates (Table 1); e_i are electron-pair transfers associated with reaction i ; E_i is concentration of enzyme allocated to reaction i , and E_T is the total protein concentration; R^C and R^N are the subset of reactions (r_i) that lead to C and N accumulation in living biomass, respectively; $f_i(S)$ are functions that describe uptake kinetics of substrate S , such as Michaelis-Menten; $\text{PAR}(t)$ is the photosynthetic active radiation; h is water column depth; and r_p is the rate of photosynthesis (Table 1). Solution of the linear programming (LP) problem gives r_i and E_i at time t , which are used in standard C and N conservation equations to obtain solution of state variables over time. See Vallino *et al.* (1996) for a more thorough development.

the various electron donor and acceptor resources that exist within the environment (Koelmans *et al.*, 2001). While this approach has been useful for well-defined laboratory experiments with a few species growing on a limited number of well-defined substrates, it is not practical for extension to more diverse microbial ecosystems with numerous or ill-defined substrates. Reductionist-based biological models fail to incorporate the governing laws that define living systems (Lawton, 1999); the models are based solely on empirical observations. Consequently, these models are brittle and often fail as the system's state changes significantly over time and space (Vallino, 2000).

To develop a robust model that can predict microbially governed biogeochemistry in spatially and temporally diverse environments, a more holistic, systems-based perspective must be taken. Our governing philosophy is that living systems synthesize and allocate metabolic capability in such a way as to optimally utilize available resources in the environment as governed by NET. What we seek to determine is the nature of the objective function that living systems tend to follow, and what causes living systems to diverge from this function.

This optimization-based approach was first developed in a thermodynamically constrained metabolic framework to examine bacterial utilization of dissolved organic matter (Vallino *et al.*, 1996). However, this model still uses an

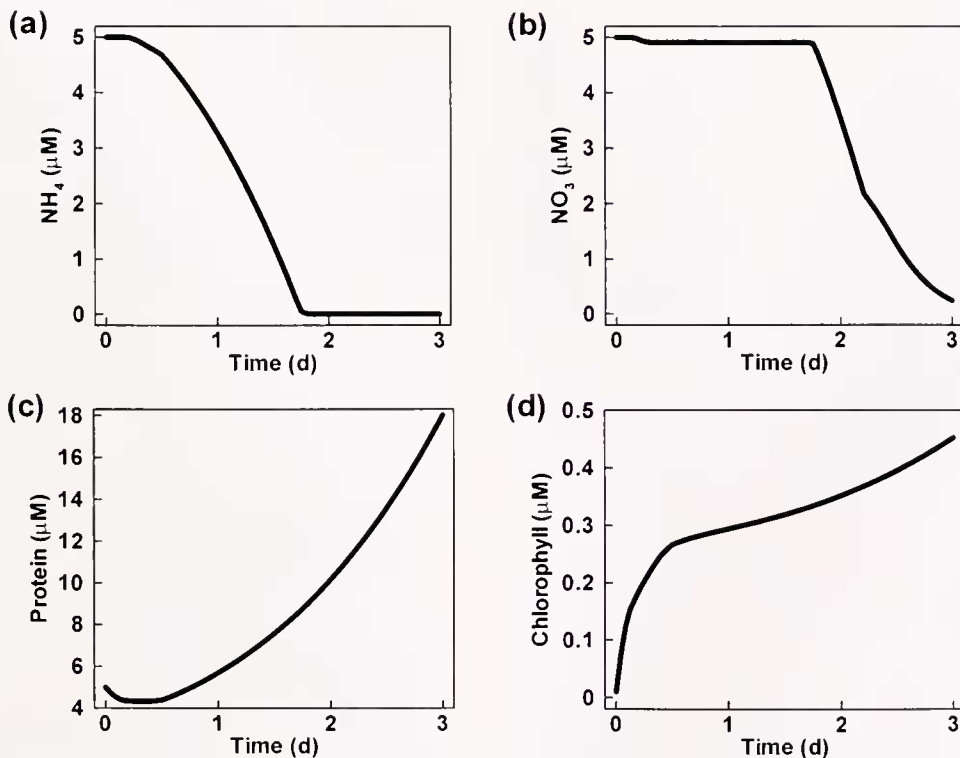


Figure 2. Metabolic model. Changes in resource concentrations of (a) ammonium and (b) nitrate, and accumulation of biological structure (c) protein and (d) chlorophyll over the course of the simulation.

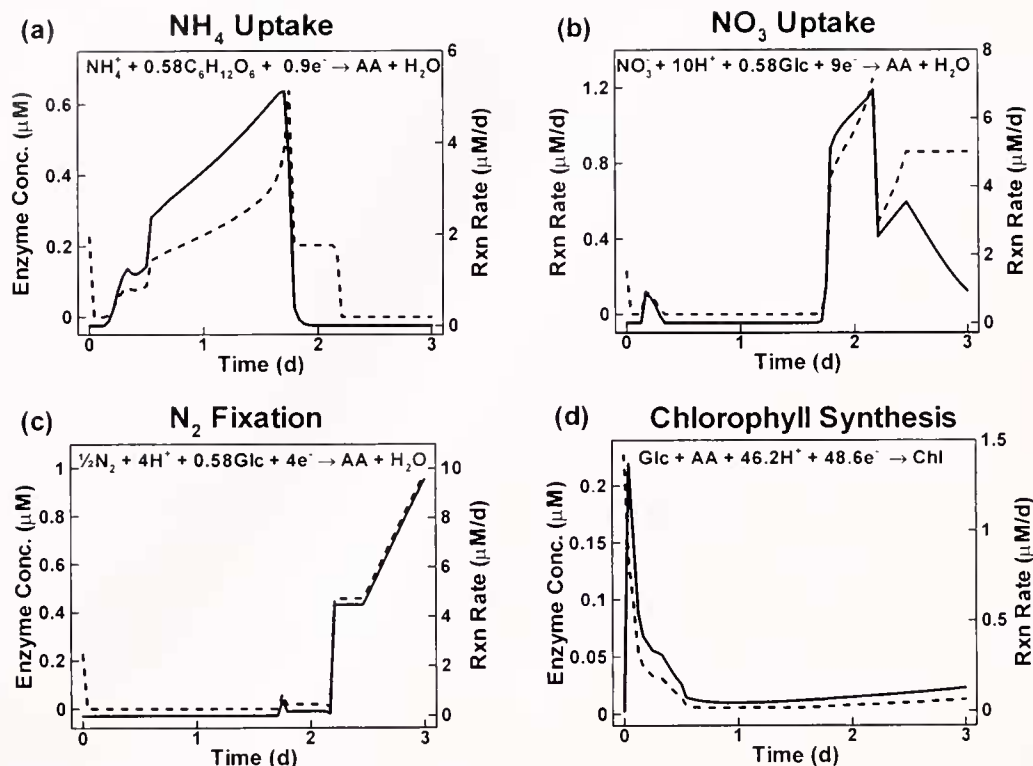


Figure 3. Reaction rates (solid line) and allocation of protein (enzyme concentration, dashed line) for reactions involving (a) ammonium uptake, (b) nitrate uptake, (c) N₂ fixation, and (d) chlorophyll synthesis during the course of the simulation. Abbreviations: Glc, glucose; AA, amino acids; Chl, chlorophyll.

organismal approach, in that the model tracks bacterial biomass. We increase the applicability of our modeling approach by removing emphasis on synthesizing bacterial biomass and placing it instead on synthesis of metabolic capability exhibited by the whole ecosystem. The model consists of a set of metabolic half-reactions that represents the major metabolic capability of a planktonic ecosystem (Table 1). But instead of synthesizing bacteria, reactions produce protein, chlorophyll, storage compounds, and other fundamental building materials observed in living systems (Fig. 1). These building materials represent those summed over all organisms in the ecosystem, not any one particular organism. Indeed, organisms are not directly modeled. Newly synthesized protein is then allocated to those metabolic reactions that optimize the specified objective criteria, while enzymes no longer in use can be degraded back into constituent amino acids (Fig. 1). A linear programming (LP) problem is used to determine the reaction rates (r_i) and enzyme concentrations (E_i) that maximize a given objective function, subject to fundamental constraints, such as energy, redox, composition, kinetics, and light-capturing capabilities (Table 2). Although the model does not distinguish species in a classic sense, it does from a functional perspective. As environmental conditions change, so do allocations of resources to metabolic reactions. Real systems accom-

plish this same objective *via* relative changes in species abundances and magnitude of gene expression.

As an example of the model, we simulate a marine phytoplankton bloom, where metabolic reactions associated with ammonium (NH₄⁺) and nitrate (NO₃⁻) uptake, N₂-fixation, carbon dioxide (CO₂) fixation, and biomass synthesis (protein and chlorophyll) are included in the model (Table 1). The optimization goal chosen was maximizing the rate of biomass synthesis, though others could be formulated. Resources made available were 5 μM NH₄⁺, 5 μM NO₃⁻, atmospheric N₂, and light. The model simulation proceeds by preferentially consuming NH₄⁺ over NO₃⁻ (Fig. 2a,b), which is evident by the allocation of protein (in the form of enzyme) to NH₄⁺ uptake (Fig. 3a), but not to NO₃⁻ uptake nor N₂ fixation (Fig. 3b,c). There is also a strong initial allocation of protein to chlorophyll synthesis (Figs. 2d, 3d), but this protein is rapidly reallocated after 0.5 d due to diminished returns on the investment in light harvesting capacity (*i.e.*, chlorophyll), which saturates at high chlorophyll concentration (Fig. 3d). As NH₄⁺ becomes exhausted (Fig. 2a), protein is reallocated from NH₄⁺ to NO₃⁻ uptake (Fig. 3a, b). Subsequently, as NO₃⁻ becomes depleted (Fig. 2b), protein is allocated to N₂ fixation (Fig. 3c).

Conclusions

If nonequilibrium thermodynamics governs biogeochemistry, our metabolic modeling approach represents a more direct means of capturing ecosystem dynamics than classic, organismal-based approaches. The approach also predicts how whole-system genomic transcription and translation should proceed, which can be compared to actual systems using techniques currently being advanced in molecular biology. Because the metabolic ecosystem model is based on fundamental governing equations, it should prove more robust and have a greater operating range than organismal-based models.

Acknowledgments

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